American Journal of Medical Genetics (Neuropsychiatric Genetics) 74:192–194 (1997)

Brief Research Communication

No Interaction Between the APOE and the Alpha-1-Antichymotrypsin Genes on Risk for Alzheimer's Disease

Danielle Fallin,* Sarah Reading, John Schinka, Jonathan Hoyne, Paul Scibelli, Michael Gold, Fiona Crawford, and Michael Mullan

Roskamp Laboratories, Institute for Research in Psychiatry, University of South Florida, Tampa, Florida

It is now known that possession of one of the three common forms of the apolipoprotein E gene (allele ε4) confers an increased risk for Alzheimer's disease (AD), both familial and sporadic, and that this risk is dose-dependent. Other genes that may play a role in AD, either through independent association with the disease or through modification of, or interaction with, the existing apolipoprotein E (APOE) risk, are now under investigation including the alpha-1-antichymotrypsin (ACT) gene, the very low density lipoprotein receptor (VLDL-R) gene, and the presenilin-1 (PS-1) gene. Kamboh et al. [1995] reported that a polymorphism in the α-1-antichymotrypsin gene could modify the risk for AD conferred by the APOE locus, specifically by increasing the risk for AD among ε4 homozygotes. The ACT gene, which is found on chromosome 14, has previously been proposed as a candidate for AD due to the presence of the ACT protein in senile plaques and the reported elevation of the protein in the cerebro-spinal fluid (CSF) and serum of AD cases. We have investigated this reported association within our familial and sporadic AD dataset, where we find no independent association between ACT and the occurrence of AD. Logistic regression analysis excludes ACT or the interaction between ACT and APOE as significant contributors in the prediction of disease status. By this analysis, ACT genotyping does not provide additional information about an individual's risk of Alzheimer's disease beyond the risk information conferred by APOE genotype alone. Am. J. Med. Genet. 74:192–194, 1997. © 1997 Wiley-Liss, Inc.

Contract grant sponsor: NIH; Contract grant number RO1-AG12412.

*Correspondence to: Danielle Fallin, 3515 E. Fletcher Ave., Tampa, FL 33613.

Received 6 May 1996; Revised 12 August 1996

KEY WORDS: Alzheimer's disease; alpha-1-antichymotrypsin; apolipoprotein E

INTRODUCTION

Since the discovery that the ϵ4 variant of the apolipoprotein E (APOE) gene is associated with an increased risk and an earlier onset of familial and sporadic late-onset Alzheimer's disease (AD) [Strittmatter et al., 1993; Poirier et al., 1993; Bennett et al., 1995], other genes have been nominated as having a similar role. In addition to the other apolipoproteins and lipoprotein receptors that have been studied [Schellenberg et al., 1987; Chartier-Harlin et al., 1994; Okuizumi et al., 1995], the alpha-1-antichymotrypsin (ACT) gene has recently been investigated for possible influence on the disease, both independently and interactively with the APOE gene [Kamboh et al., 1995].

Kamboh et al. [1995] reported an independent association of the ACT gene to AD in the absence of APOE information. Perhaps more importantly, they also reported an interaction in the risk conferred by the APOE ϵ4 allele and ACT genotype, specifically in that ACT genotype significantly modifies the risk conferred by the presence of the APOE ϵ4 allele. Alpha-1-antichymotrypsin has been studied for association with the AD process because of its presence in senile plaques, its ability to bind to the Aβ peptide [Fraser et al., 1993], its ability to induce Aβ fibril formation [Ma et al., 1994], its role as a protease inhibitor, and reports that levels of ACT are elevated in both the CSF and serum of AD patients [Licastro et al., 1995]. Kamboh et al. [1995] genotyped subjects at a di-allelic polymorphism in the signal peptide coding region of the gene (a polymorphism coding for either threonine [T allele] or alanine [A allele]), and found a significant but small increase in the frequency of the A allele among AD cases over controls (57% versus 51%). They further reported that the risk (odds ratio) for those carrying an APOE ϵ4 in the ACT-AA group was greater than the

TABLE I. APOE Allele Frequencies and Odds Ratio per E4 Allele Dose

	AD (n = 186)	Controls (n = 222)	Odds ratio for carrying ε4	$P <$ value
Allele frequencies				
ε2	.059	.059	–	
ε3	.548	.775	–	
ε4	.392	.167	–	0.00001
APOE ε4 dose	(n = 93)	(n = 111)		
no ε4	37%	68%	1	
one ε4	48%	30%	3.05 (1.67–5.58)	0.0004
ε4, ε4	15%	2%	15.6 (3.4–75.5)	0.0005

ϵ4-conferred risk in the overall data set, suggesting that the A allele modifies the effect of the APOE ϵ4 allele. Their results suggest a substantive increase in the prediction of AD with the addition of knowledge of the ACT genotype.

In this study, we attempted to replicate the Kamboh findings by genotyping our AD samples at the ACT and APOE loci (using primers and experimental conditions as previously described by Wenham, et al., [1991] and Kamboh et al. [1995]). Ten cases from a British late-onset familial dataset as well as 83 subjects recruited from the University of South Florida and Mount Sinai Memory Disorder Clinics were genotyped and compared to 112 controls recruited from a population-based screening study in the Miami area (mean age of controls = 73.0; S.D. = 7.1). Of the nonfamilial cases, 46% were recruited as part of the population-based screen, and 54% were self-referring to the clinics. All 93 cases met NINCDS-ADRDA criteria for probable or possible AD, and the average age of onset was 72.91 (S.D. = 7.41). In cases where familial samples were used, only one member per family was included in the analyses.

APOE Risk

The data were first examined for association of APOE to AD to confirm that the genetic composition of our dataset is comparable to that of other published studies (Table I). Our analyses showed the expected association between the ϵ4 allele and AD, with the ϵ4 frequency increasing from 0.17 among controls to 0.39 among AD cases (significant at $P < 0.00001$), and logistic regression analysis showed a 3.05 odds ratio (OR; 95% confidence interval [CI] = 1.67–5.58) for carrying one copy of the ϵ4 allele, and a 15.6 OR (CI = 3.37–75.5) for carrying two copies of ϵ4 (Table I).

ACT Risk

The analysis for association of ACT and AD failed to detect a significant difference in allele frequencies between cases and controls, with an A allele increase from 48% among controls to only 52% among cases ($P = 0.56$, Table II). Further, the odds ratio for carrying one A (genotype AT) was 0.88 (CI = 0.45–1.71), and the OR for carrying two (AA) was only 1.3 (CI = 0.61–2.81), which was not statistically significant. Table II summarizes these results, showing no association between ACT and AD in any of the analyses performed.

Predictive Value of APOE and ACT

To examine the possible interaction between APOE and ACT in contributing to AD, we employed logistic regression analysis. In order to examine the relative contribution of APOE ϵ4 allele dose, ACT genotype (or A allele dose), and the APOE by ACT interactions, we conducted the regression analysis using a forward stepwise entry procedure. In this procedure, variables enter the logistic regression equation in order of size of contribution. The Wald statistic, set at a probability value of .05, was used as a criterion to determine whether a variable would enter the analysis. At the first step of the analysis, APOE entered the equation ($\chi^2 = 26.84$, $P < .0001$). This model, employing only APOE genotype in the prediction of affected status (AD versus non-AD), resulted in a positive prediction value of 66.18%. In the second step of the analysis, neither ACT genotype nor the APOE by ACT genotype interaction met the criteria for entry into the regression equation. This result indicates that, once the pre-

TABLE II. Act Genotypes and Allele Frequencies and Odds Ratio per Allele A

	AD (n = 186)	Control (n = 224)	Odds ratio for carrying A	P value
Allele Frequencies				
A	.516	.482		
T	.484	.518		0.56
ACT genotype	(n = 93)	(n = 112)		
TT	25.8%	25.9%	1.0	
AT	45.2%	51.8%	0.88 (0.45–1.71)	0.6965
AA	29.0%	22.3%	1.31 (0.61–2.81)	0.4964

dictive power of APOE genotype has been accounted for, no further significant information is added to the model by the inclusion of ACT genotype or the APOE by ACT genotype interaction.

In order to specifically examine the absolute increase in predictive power of the ACT genotype and/or the APOE by ACT genotype interaction with relation to prediction provided by APOE alone, we conducted a second logistic regression analysis employing simultaneous entry of all variables into the model. The results of this analysis are presented in Table III, and show only APOE ϵ4 dose to be a significant predictor of disease status. Values of the Wald statistic for ACT genotype and the APOE by ACT genotype interaction do not approach significance at even the 0.10 level. This model, including contributions from all variables in the prediction of affected status, resulted in a positive prediction value of 66.18%. This result shows no improvement in prediction accuracy over the model that includes APOE genotype alone.

Our results fail to support the findings of Kamboh et al. [1995] We find no association between ACT genotype and risk for AD. Additionally, our analyses fail to support modification of APOE risk by ACT genotype. The addition of ACT genotype does not appear to add information about an individual's risk for AD beyond the information provided by APOE genotype.

ACKNOWLEDGMENTS

This work was funded in part by Mr. and Mrs. Robert Roskamp as well as NIH grant RO1-AG12412.

TABLE III. Summary of Logistic Regression for Diagnosis

Variable	Wald	df	*P* value
APOE	12.019	2	0.0025
ACT	0.2914	2	0.8644
ACT*APOE	2.8248	4	0.5876
Constant	1.7591	1	0.5936

REFERENCES

Bennett C, Crawford F, Osborne A, Diaz P, Hoyne J, Lopez R, Roques P, Duara R, Rossor M, Mullan M, (1995): Evidence that the APOE locus influences rate of disease progression in late onset familial Alzheimer's disease but is not causative. AJMG 60:1–6.

Chartier-Harlin MC, Parfitt M, Legrain S, Perez-Tur J, Brousseau T, Evans A, Berr C, Vidal O, Roques P, Gourlet V, Fruchart JC, Delacourte A, Rossor M, Amouyel P, (1994): Apolipoproetin E ϵ4 as a major risk factor for sporadic early and late onset Alzheimer's disease: Analysis of the 19q13.2 chromosomal region. Hum Mol Genet, 3:569–574.

Fraser PE, Nguyen JT, McLachlan DR, Abraham CR, Kirschner DA, (1993): Alpha-1-antichymotrypsin binding to Alzheimer A beta peptides is sequence specific and induces fibril disaggregation in vitro. J Neurochem 61:298–305.

Kamboh M, Sanghera D, Ferrell R, DeKosky S, (1995): APOE 4-associated Alzheimer's disease risk is modified by alpha-1-antichymotrypsin polymorphism. Nature Genet 10:486–488.

Licastro F, Parnetti L, Morini MC, Davis LJ, Cucinotta F, Gaiti A, Senin U, (1995): Acute phase reactant alpha-1-antichymotrypsin is increased in cerebrospinal fluid and serum of patients with probable Alzheimer's disease. Alzheimer's Dis Assoc Disor 9:112–118.

Ma J, Yee A, Brewer HB Jr, Das S, Potter H, (1994): Amyloid-associated proteins alpha-1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer's beta-protein into filaments. Nature 372:92–94.

Okuizumi K, Onodera O, Namba Y, Kazuhiko I, Yamamoto T, Seki K, Ueki A, Nanko S, Tanaka H, Takahashi H, Oyanagi K, Mizusawa H, Kanazawa I, Tsuji S, (1995): Genetic association of the very low density lipoprotein (VLDL) receptor gene with sporadic Alzheimer's disease. Nature Genet 11:207–209.

Poirier J, Davignon J, Bouthillier D, Kogan S, Bertrand P, Gauthier S, (1993): Apolipoprotein E polymorphism and Alzheimer's disease. Lancet 342:697–699.

Relkin N, NIA/AA Conference Invited Researchers (1996): Apolipoprotein E genotyping in Alzheimer's disease. Lancet, 347:1091–1095.

Sambrook J, Fritsch E, Maniatis T, (1989): "Molecular Cloning: A Laboratory Manual." New York: Cold Spring Harbor Laboratory Press.

Schellenberg G, Deeb S, Boehnke M, Bryant E, Martin G, Lampe T, Birs T, (1987): Association of an apolipoprotein CII allele with familial Alzheimer's dementia of the Alzheimer type. J Neurogenet 4:97–108.

Strittmatter D, Saunders A, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses A, (1993): Apolipoprotein E: High-avidity binding to b-amyloid and increased frequency of type 4 allele in late onset Alzheimer's disease. Proc Natl Acad Sci 90:1977–1981.

Wenham PR, Blandell G, Price WH, (1991): Apolipoprotein genotyping by one-step PCR. Lancet 50:1158–1159.